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Role of Stat3 in suppressing anti-tumor immunity

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Abstract

Recent evidence suggests that what sustains a tumor's survival and proliferation can also subvert the immune system from its defense role. As a point of convergence for numerous oncogenic signaling pathways, Stat3 is constitutively activated in diverse human cancer cells. Activated Stat3 not only upregulates genes critical for survival, proliferation, angiogenesis and metastasis, it also promotes expression of immune suppressive factors while inhibiting Th1 immunostimulatory molecules. By virtue of its ability to promote expression of many factors that activate Stat3 in diverse cells, Stat3 allows malignant and immune cells resonate, forming close partnership for tumor immune evasion, tumor progression and resistance to therapies.

Introduction

An unexpected observation made during a tumor gene therapy experiment[1] initiated a persistent pursuit for a potential role of Stat3 in tumor immune suppression. Results from these studies and others reveal that oncogenic signaling pathways, many of which converge on Stat3, are critical for coordinating and maintaining immune suppression (for review, see[2]). Stat3 activation, which occurs in both tumor cells and tumor-interacting immune cells, not only inhibits expression of Th1 mediators but also promotes production of diverse immunosuppressive factors[3,4]. A critical role of Stat3 in mediating the crosstalk between tumor cells and tumor-interacting immune cells has been reviewed in depth elsewhere[2]. In this review we will provide a brief summary of the milestones in our understanding of Stat3 in tumor immune evasion and suppression. We will devote much of this review on recent findings that suggest the involvement of Stat3 in tumor-induced regulatory T cell accumulation, and in regulating Th17 cells, which is critical for inflammation and may have a potential role in cancer development. We will conclude this review by summarizing recent human studies suggesting that Stat3 is a promising target for enhancing efficacies of immunotherapies.

Oncogenesis and suppression of tumor immunity: the role of Stat3

An important role of Stat3 in cancer initiation and development has been underscored. Stat3 is both a point of convergence of numerous most commonly activated oncogenic pathways and a transcriptional regulator of diverse tumor-promoting factors[5]. Stat3 was originally discovered in the context of cytokine signaling and later growth factor signaling. Although STAT proteins are usually latent in the cytoplasm and their activation is tightly controlled by negative regulators including SOCS and PIAS proteins as well as phosphatases, in oncoprotein-

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transformed cells and in diverse cancer cells Stat3, and to a lesser extent, Stat5, is constitutively activated. The constitutive activation of Stat3 in cancer is due to the fact that receptor signaling of many of cytokines and growth factors, for examples, interleukin 6 (IL-6) and interleukin 10 (IL-10), EGF, HGF, Her2/Neu, VEGF, is overly activated in cancer. In addition, oncoproteins, such as Src, which is often active in several types of human cancer, are also Stat3 activators. Stat3 itself has also tumorigenic properties and in many ways can be viewed as an oncogene [5,6]. Stat3 has been shown to be anti-apoptotic and mitogenic in that it has the capacity to upregulate expression of a number of genes critical for survival and proliferation. It has also been shown that Stat3 inhibits several pro-apoptotic and/or anti-proliferative genes. Importantly, the role of Stat3 as a critical transcriptional factor for upregulating angiogenic and metastatic genes has also been demonstrated. The ability of Stat3 in transducing the signals from numerous oncogenic signaling pathways and in regulating expression of diverse genes in favor of tumorigenesis underlies its fundamental role in cancer development[5].

Significant progress made within the recent years in the field of tumor immunology highlighted the importance of tumor immune evasion and immunosuppression in malignant progression [7,8]. Nevertheless, how tumor cells impact anti-tumor immunity at the molecular level is less well understood. A serendipitous observation that targeting Stat3 in tumors involves bystander tumor cell killing associated with infiltration of various immune effector cells[1] fueled the search for the underlying molecular and cellular mechanism(s). These studies along with many other investigations have led to the discovery that Stat3 inhibits expression of several Th1 immune mediators, both in mouse and human tumor cells[3,9,10]. The ability of Stat3 to inhibit expression of similar immunostimulatory molecules in immune cells in tumor setting and in normal cells has also been demonstrated[4]. The underlying molecular mechanism(s) for downregulating Th1 immune responses remain fully unexplored, although it has been suggested that Stat3 has an antagonistic effect on immunostimulatory effects of NF- κ B[11, 12] and Stat1[13], both of which are critical for inducing Th1 immune responses.

A critical aspect of Stat3's role in suppressing tumor immunity is that Stat3 upregulates expression several immunosuppressive factors[2]. One of them is IL-10, which has been demonstrated to affect negatively Th1 immune responses at multiple levels[14,15]. VEGF, too, is well known for its inhibitory impacts on dendritic cell maturation/activation[16]. These Stat3-regulated tumor factors activate Stat3 in dendritic cell progenitors and in other immune cells including both innate immune cells and T cells[4]. Myeloid cells interacting with tumor cells display constitutively activated Stat3 and have been shown to have an inhibitory effect on antitumor immunity (for reviews, see [2,17]). Because many Stat3 target genes encoding factors that can also activate Stat3 in various cells, a feed-forward mechanism for constitutive activation of Stat3 in both tumor cells and tumor-interacting normal cells is entailed. This list of factors – both Stat3 regulated and Stat3 regulator – continues to grow.

Role of Stat3 in regulatory T cells

CD4⁺ regulatory T cells (Treg) suppressing antitumor immunity has been shown in mouse models, and Treg cells are linked to poor prognosis for cancer patients[18,19]. While Tregs comprise only 5–10% of total CD4-positive lymphocytes residing in peripheral lymphoid organs under normal conditions, antigen-specific Treg cells can be induced during tumor progression. First indication that Stat3 activity in the tumor stroma contributes to tumor Treg accumulation was provided by a study involving inducing ablation of Stat3 in hematopoietic cells in tumor-bearing mice[4]. Mice lacking Stat3 in myeloid compartment of tumor stroma, including DCs and macrophages, showed reduced numbers of tumor-infiltrating CD4⁺CD25⁺/Foxp3⁺/Lag-3⁺ Tregs, which was accompanied by increased CD8⁺ effector T cells. An important role of TGF β in mediating Treg expansion through direct interaction of CD4⁺CD25⁺ cells with DCs has been demonstrated in various systems[20,21]. In addition,

resident tumor DCs might also actively promote immunosuppression through expansion of Tregs[22]. Induction of FoxP3⁺ Tregs *in vivo* requires direct contact of CD4⁺CD25⁻ T cells with DCs for TCR ligation and costimulation with exogenous or endogenous TGFβ [23,24]. Tumor progression indeed correlates with the accumulation of TGFβ-secreting immature myeloid DCs which promote expansion of existing Tregs within tumor-draining lymph nodes [25]. A recent study by Yoshimura and colleagues sheds some light on the molecular mechanisms regulating tolerogenic potential of DCs[26]. Ablation of the negative regulator of Stat3 signaling, SOCS3 (Suppressor of Cytokine Signaling 3) in DCs induced persistent Stat3 activation, enhanced secretion of TGFβ. Nevertheless, it is important to note that DCs produce only low levels of TGFβ which are not sufficient to induce Treg generation *in vitro*. Additional sources of TGFβ or other effects such as reduced expression of MHC class II and costimulatory molecules due to constitutive Stat3 activation in tumor-residing DCs contribute to expansion of tumor-infiltrating Foxp3⁺ T cells[4].

Given that Stat3 contributes to upregulation of TGFβ and IL-10 [27,28], two major immunosuppressive mediators secreted by Tregs, intrinsic Stat3 signaling may regulate function of CD4⁺FoxP3⁺ Tregs. However, induction of FoxP3 expression by IL-2 in CD4⁺CD25⁺ cells is Stat5- and not Stat3-dependent, even though binding sites for both transcription factors were found in the FoxP3 promoter[29,30]. Stat5 knock-out mice show dramatic reduction in the number of FoxP3-expressing Tregs, whereas Stat3 ablation in CD4⁺ T cells does not affect Treg numbers but instead it prevents IL-6-mediated attenuation of FoxP3 expression[30]. Similarly, SOCS3^{-/-}CD4⁺ T cells with constitutive Stat3 activity are associated with Th3-like differentiation and increased expression of TGFβ and IL-10 but not FoxP3[27]. Nevertheless, Stat3 might contribute Treg expansion in tumor by inducing FoxP3 expression in T lymphoma cells transformed by oncogenes like nucleophosmin/anaplastic lymphoma kinase (NPM/ALK)[31]. It still remains to be determined if tumor-induced Stat3 activation could enhance tolerogenic function of Treg recruited into the tumor bed from the periphery.

Stat3 and Th-17 immunity: a possible role in suppressing tumor immune responses

While regulatory T cells represent the largest population of CD4⁺ lymphocytes in progressing tumors, subpopulations of tumor-infiltrating CD4⁺ and also CD8⁺-positive T cells capable of secreting IL-17 have been recently described [32]. IL-17-positive T cells accumulate in parallel with Tregs, although at several times lower frequency, within tumor tissues in mice as well as in blood and ascites from ovarian carcinoma patients. Interestingly, in contrast to Tregs, IL-17⁺ T cells were absent from tumor-draining lymph nodes. It is tempting to ask if IL-17⁺ CD4⁺ and CD8⁺ cells instigate chronic inflammatory conditions within tumor bed similar to the function of Th17 cells in the pathogenesis of autoimmune diseases[33,34]. It seems plausible given the fact that IL-23, a critical mediator for Th17-CD4⁺ cell generation, was implicated in tumor-associated inflammation, angiogenesis and immunosuppression[35]. Myeloid cells, including macrophages, migrating into the tumor from peripheral lymphoid organs are major source of IL-23p19. IL-23p19 expression in the tumor milieu is Stat3 dependent (Kortylewski M., Xin H. and Yu H., unpublished data). Rapidly increasing number of studies indicates the critical role of Stat3 in the multi-step process of Th17 generation[36–42]. Activation of Stat3 by IL-6 with TGFβ costimulation, is required to initiate Th17 differentiation and induce expression of IL-21[39,41]. IL-21 in turn acts as an autocrine factor sustaining Stat3 activation and promoting expression of RORγt which upregulates IL-23R expression. At this stage cells finally acquire ability to respond to IL-23 signals, leading to Stat3-dependent induction of IL-17[36,40]. While there is some evidence that IL-23 may have a role in tumor immune suppression/evasion, the outcome of Th17 accumulation in tumor microenvironment may depend on the fine balance with other T cell populations especially tumor-infiltrating Tregs.

Intratumoral injections of DCs overexpressing IL-23 or systemic administration of IL-23 at high levels was shown to actually induce antitumor immunity[43,44]. A role of Th17 CD4⁺ T cells in tumor immune suppression or chronic inflammation-associated carcinogenesis, and the involvement of Stat3 in such a process, remains to be fully explored.

Evidence of Stat3 as an important target for cancer immunotherapy in humans

Mice with induced deletion including the tyrosine 705 in Stat3 allele(s) in the hematopoietic system or macrophages exhibit enhanced Th1 activity and can suffer from chronic enterocolitis [11,45,46]. Consistent with the findings in mouse studies, an indication that Stat3 can inhibit pro-inflammatory signals in human is provided by recent studies identifying mutations in Stat3 in patients with hyper-IgE syndrome (HIES)[47,48]. Several discrete mutations predicted to directly affect the DNA-binding and SH2 domain responsible for dimerization correlate with high production of proinflammatory cytokines including TNF α , IL-12 and IFN γ in leukocytes from HIES patients[48]. As in murine tumor cells, Stat3 in human melanoma cells not only promotes expression of immunosuppressive factors, it also inhibits immunostimulatory factors [10]. In addition to Stat3, an involvement of BRAF-MAPK in immune suppression has also been indicated in human melanoma cells[10]. As evidence accumulating in mouse models that inhibiting Stat3 can boost the efficacies of immunotherapy, supporting data from clinical trials are also emerging. A recent study of prospective neoadjuvant trial of interferon- α therapy suggests that higher phosphorylated Stat1 (pStat1) over phosphorylated Stat3 in both tumor cells and lymphocytes pretreatment were associated with longer overall survival[49]. In addition, high density interferon- α treatment is associated with upregulation of pStat1 and down-regulation of pStat3[49]. These data suggest that the ratio between pStat1/pStat3 at the baseline could serve as a predictor of clinical outcome of certain immunotherapies and that increasing this ratio may tip the balance towards better immunotherapeutic effects. Consistent with these findings, a recent study involving patients with metastatic renal cell carcinoma suggests that Stat3 polymorphism predicts interferon- α response[50]. Proof-of-concept experiments in cultured cells support that higher expression of Stat3 due to polymorphism can result in resistance to interferon therapy. While several studies in mice suggest that Stat3 targeting can improve the outcome of various immunotherapeutic approaches, due to lack of clinical grade Stat3 inhibitors, no human trials using direct Stat3 targeting for enhancing immunotherapy have been conducted to date.

Conclusion

A point of convergence for numerous oncogenic signaling pathways, Stat3 is constitutively activated in diverse cancers, promoting tumor cell survival, proliferation, cancer angiogenesis and metastasis. Recent studies have also shown that Stat3 is activated in tumor stromal immune cells. Activated Stat3 promotes expression of immunosuppressive factors while inhibiting Th1 immunostimulatory molecules. Much remains to be learned about Stat3's role in mediating tumor Treg expansion. The importance of Th17 T cells, whose differentiation is Stat3-dependent, in promoting tumorigenesis needs also to be defined. Nevertheless, Stat3 has emerged as an important target for cancer immunotherapy, either alone or in conjunction with other promising immunotherapeutic approaches. The key is now to discover specific and effective drugs, be it small-molecule or siRNA-based, that can be safely used in humans.

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Figure. Role of Stat3 in tumor immune suppression

Stat3 is constitutively activated in tumor cells of diverse origin. Activated Stat3 in tumor cells promotes production of factors, including but not limited to VEGF and IL-10. These tumor factors in turn activate Stat3 in various immune subsets, such as tumor associated macrophages (TAM), immature DCs (ImDC), neutrophils and NK cells. When Stat3 is activated in these immune cells, their production of immunostimulatory molecules is reduced and their ability to kill or inhibit tumor cell growth compromised. Many of these immune cells also produce immunosuppressive factors, including but not limited to IL-10, TGF- β , IL-23, in a Stat3-dependent manner, which is accompanied by an increase in tumor Treg cells, and potentially Th-17 cells. While a clear role of Treg in tumor immune evasion has been well documented, to what extent Th-17 cells contribute to tumor immune suppression remains further explored.